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Abstract

Background: In support of the Integrated Risk Information System (IRIS), the US Environmental Protection Agency (EPA) completed a Toxicological Review of Tetrachloroethylene (Perchloroethylene) in February, 2012.

Objectives: This article reviews key findings and scientific issues regarding the human health effects of tetrachloroethylene in EPA's Toxicological Review.

Methods: This assessment synthesized and characterized a substantial database of epidemiologic, experimental animal, and mechanistic studies. Key scientific issues were addressed through modeling of tetrachloroethylene toxicokinetics, synthesis of evidence from neurological studies, and analyses of toxicokinetic, mechanistic, and other factors (tumor latency, severity, and background rate) in interpreting experimental animal cancer findings. Considerations in evaluating epidemiologic studies included the quality (e.g., specificity) of the exposure assessment methods and other essential design features, and the potential for alternative explanations for observed associations (e.g., bias or confounding).

Discussion: Toxicokinetic modeling aided in characterizing the complex metabolism and multiple metabolites that contribute to tetrachloroethylene toxicity. The exposure assessment approach was a key evaluation factor for epidemiologic studies of bladder cancer, non Hodgkin lymphoma, and multiple myeloma, which provided suggestive evidence of carcinogenicity. Bioassay data provided conclusive evidence of carcinogenicity in experimental animals. Neurotoxicity was identified as a sensitive non-cancer health effect, occurring at low exposures, a conclusion supported by multiple studies. Evidence was integrated from human, experimental animal, and mechanistic datasets in assessing adverse health effects of tetrachloroethylene.

Conclusions: Tetrachloroethylene is likely to be carcinogenic to humans. Neurotoxicity is a sensitive adverse health effect of tetrachloroethylene.

Introduction

Tetrachloroethylene (perchloroethylene) is a widely used chlorinated solvent that is produced commercially for use in dry cleaning, textile processing, and metal-cleaning operations. Tetrachloroethylene has been detected in drinking water, indoor environments, ambient air, groundwater, and soil. Many point sources of contamination exist in the US (U.S. EPA 2013c) and tetrachloroethylene is also commonly found at Superfund hazardous waste sites (U.S. EPA 2013b). Regarding exposure to the general population, the Centers for Disease Control and Prevention (CDC 2013) reports that tetrachloroethylene levels assessed in the most recent biomonitoring survey (2003-2004 subsample of the National Health and Nutrition Examination Survey, NHANES) appear similar or slightly lower than levels reported in earlier NHANES surveys. The primary exposure routes are via inhalation, including as a result of vapor intrusion from contaminated soil and water (U.S. EPA 2013d), and ingestion of contaminated water.

EPA identified tetrachloroethylene as a priority existing chemical for regulatory action review under the Toxic Substances Control Act (U.S. EPA 2012c), and as one of several volatile organic compounds to be regulated as a group in drinking water (U.S. EPA 2010). Supporting these and other Agency actions, EPA's Integrated Risk Information System (IRIS) program released an updated human health assessment of tetrachloroethylene in February, 2012 that included an extensive Toxicological Review (U.S. EPA 2012b). The Toxicological Review was developed according to the general guidelines for risk assessment set forth by the National Research Council (NRC 1983, 1994), as well as relevant EPA Guidelines and Risk Assessment Forum technical panel reports (U.S. EPA 2013a). The literature search strategy was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Primary

peer-reviewed literature published during or before August 2011 was included. Public submissions to EPA and peer-reviewed information (including health assessments developed by other organizations, review articles, and independent analyses of the health effects data) were also considered for inclusion. Toxicokinetic, mechanistic and other data (tumor latency, severity, and background rate) were considered in interpreting experimental animal cancer findings. Individual study evaluation considered essential design features (particularly, the study species and population, and the relevance of the exposure paradigm); other considerations are discussed in the following sections. The Toxicological Review (U.S. EPA 2012b) provides additional detail regarding the source literature databases, the relevant EPA guidance, and the study evaluation criteria.

The assessment development involved multiple internal and external peer review stages. Critical input was provided by a 2004 peer consultation workshop on tetrachloroethylene neurotoxicity (U.S. EPA 2004), a 2010 National Research Council (NRC) panel report (National Research Council 2010), a 2011 peer review of the physiologically based pharmacokinetic (PBPK) model applications (U.S. EPA 2012b), and written and oral comments from scientists within EPA, other federal agencies, the Office of Management and Budget (OMB) (U.S. EPA 2012a), and the public (Regulations.gov 2008). This article describes key findings and scientific issues addressed in EPA's 2012 Toxicological Review of tetrachloroethylene, covering the following topics:

(1) the role of metabolism in toxicity, informed by the development and application of an updated PBPK model;

(2) the carcinogenicity of tetrachloroethylene, based on analyses of epidemiological studies, multiple laboratory animal bioassays, and mechanistic data;

(3) non-cancer toxicity focusing on neurotoxicity as a sensitive outcome.

Role of Metabolism in Tetrachloroethylene Toxicity

PBPK models can aid in integrating complex toxicokinetic information on absorption, distribution, metabolism, and excretion of environmental chemicals and their metabolites. These models are constructed from physiologic information as well as chemical- and metabolite-specific toxicokinetic data. Some models separate datasets for model calibration and evaluation, utilizing Bayesian methods to strengthen model predictions. PBPK models are used in human health assessments to predict the extent and nature of metabolism across species or exposure routes.

The metabolism of tetrachloroethylene yields multiple metabolites through two main irreversible pathways: oxidation via the microsomal mixed-function oxidase system (i.e., cytochrome P450s) and conjugation with glutathione (GSH) by glutathione *S*-transferases (GSTs) (Lash and Parker 2001) (see Figure 1). Oxidation occurs predominantly in the liver to a Fe-O intermediate, the primary fate of which is thought to be trichloroacetyl chloride (TCAC), which then hydrolyses to yield trichloroacetic acid (TCA). A secondary fate of oxidation is the epoxide (tetrachloroethylene-O), which decomposes to ethandioyl dichloride (EDD) and then to CO and CO_2 (Yoshioka et al., 2002). Oxalic acid has been reported as both an *in vivo* and *in vitro* product of tetrachloroethylene oxidation (Pegg et al., 1979; Yoshioka et al., 2002), and may either be derived from the epoxide or directly from the Fe-O intermediate. Tetrachloroethylene conjugation with GSH in the liver or kidney forms trichlorovinyl glutathione (TCVG), which is further processed in the kidney, forming the cysteine conjugate *S*-trichlorovinyl-L-cysteine (TCVC). TCVC may be bioactivated by beta-lyase or flavin-containing monooxygenases to

reactive species (Anders et al. 1988; Krause et al. 2003), or (reversibly) undergo *N*-acetylation to the mercapturate N-acetyl trichlorovinyl cysteine (NAcTCVC). NAcTCVC is then excreted in urine or sulfoxidated by CYP3A to reactive species (Werner et al. 1996). Dichloroacetic acid (DCA), excreted in urine, is thought to be an end product of beta-lyase-mediated bioactivation (Lash and Parker, 2001), although a small contribution from TCA dechlorination cannot be ruled out.

Tetrachloroethylene liver effects are thought to result from oxidative metabolites (Buben and O'Flaherty 1985), whereas metabolites resulting from GSH conjugation are hypothesized to cause kidney effects (Lash and Parker 2001). The identity of tetrachloroethylene metabolites involved in the induction of other tetrachloroethylene health effects is less clear, although tetrachloroethylene itself has been presumed to cause neurological effects (e.g., Boyes et al., 2009).

Many PBPK models for tetrachloroethylene have been developed to predict the relationship between external measures of exposure and internal dose (Chen and Blancato (1987); Reitz et al. (1996); Rao and Brown (1993); Gearhart et al. (1993); Clewell et al. (2005); Loizou (2001); Chien (1997); Bois et al. (1996) [updated by Chiu and Bois (2006)], Covington et al. (2007) [updated by Qiu et al. (2010)]. These PBPK models have led to a wide range of predictions for the amount of tetrachloroethylene metabolized in humans at low exposure levels, with estimates across the various models spanning an order of magnitude or more (Chiu and Ginsberg, 2011).

In an attempt to reconcile these uncertainties, Chiu and Ginsberg (2011) developed a "harmonized" PBPK model that integrated the previous models and data. The harmonized model predicted oxidative metabolism with fairly high confidence. However, estimates of the extent of

GSH conjugation in humans were substantially more uncertain, spanning more than three orders of magnitude. These predictions provide a plausible explanation for apparently inconsistent findings among previously published models. In particular, previously conducted analyses that concluded low total metabolism (roughly 1% of tetrachloroethylene uptake) also assumed that TCA (derived from oxidative metabolism) represented 30−100% of total metabolism [e.g.,(Chen and Blancato 1989; Clewell et al. 2005; Covington et al. 2007; Qiu et al. 2010)]. These results are consistent with the Chiu and Ginsberg (2011) model predictions that *oxidative* metabolism is low in humans. On the other hand, previous analyses that concluded greater metabolism (>20% of tetrachloroethylene uptake) either allowed for both oxidative metabolism and GSH conjugation, or made inferences based on disappearance of the parent compound [e.g., (Bois et al. 1990; Bois et al. 1996; Chiu and Bois 2006; Reitz et al. 1996; Ward et al. 1988)]. These results are consistent with the Chiu and Ginsberg (2011) model predictions that the amount of *GSH conjugation* metabolism in humans is highly uncertain, and might be high, low, and/or highly variable.

Carcinogenicity

Evaluation of cancer epidemiology data

Much of the epidemiological research has been conducted in the dry cleaning industry, in which tetrachloroethylene was widely used from 1960 onward in the United States and Europe. A recent comprehensive review of 109 occupational studies with exposure measures estimated a mean exposure of 59 ppm in dry cleaning workers based on personal measurements (Gold et al. 2008). These measures varied considerably based on type of work, however, ranging from < 10 ppm for spotters, pressers, and counter clerks to > 100 ppm for machine operators.

Exposures in metal and plastic degreasing industries were also high (mean of approximately 100 ppm).

A key consideration in the evaluation of these studies was the quality (e.g., the specificity) of the exposure assessment methods. The ability of a study to identify cancer hazards is strengthened by "higher quality or higher specificity" exposure-assessment approaches that allow for delineation of exposure potential to individual subjects. In particular, these exposure-assessment methodologies included: 1) biological monitoring data; 2) cohort studies with job exposure matrix based on historical industrial monitoring data; 3) case-control studies with using a job exposure matrix focusing on tetrachloroethylene based on information on job title and tasks or duties; 4) additional sources of information such as union records or modules for specific jobs; and 5) studies of residential tetrachloroethylene exposure using a statistical model of water distribution system to estimate delivered dose to a subject's home (see also Table 1). Because of the variability in exposure potential within dry cleaning occupations, less specific exposure-assessment approaches (e.g., through broader job title groups or plant-or-geographically based classifications) were given less weight but were not excluded from the hazard evaluation. Other study quality considerations included lack of support for alternative explanations for observed associations (e.g., bias or confounding).

The epidemiologic evidence from cohort and case-control studies provides evidence of associations between tetrachloroethylene exposure and bladder cancer, non-Hodgkin lymphoma and multiple myeloma in adults. Of these, bladder cancer and non-Hodgkin lymphoma were considered to have the strongest databases based on the relative consistency of observed

association among studies with the higher quality exposure measurement and indication of increasing risk with increasing exposure among the studies using a cumulative exposure metric.

Cohort and case-control studies of bladder cancer, non-Hodgkin lymphoma and multiple myeloma using a higher quality exposure assessment methodology are summarized in Table 1; summaries of the other cancer sites can be found in the Toxicological Review (U.S. EPA 2012b). Studies of dry cleaners, launderers, and pressers used additional information to distinguish tetrachloroethylene-exposed from other workers (Blair et al. 2003; Calvert et al. 2011; Lynge et al. 2006; Pesch et al. 2000; Seldén and Ahlborg 2011). Specificity was also improved in studies in other work settings and in population-based case-control studies that used an individual-level exposure assignment (e.g., through a job exposure matrix, tetrachloroethylene in blood as a biological marker, or a statistical model of water distribution to estimate a delivered tetrachloroethylene dose to a subject's home (Anttila et al. 1995; Aschengrau et al. 1993; Boice et al. 1999; Gold et al. 2010; Miligi et al. 2006; Radican et al. 2008; Seidler et al. 2007). Smoking history was considered a potential confounder only in the bladder cancer studies, because it is not a known risk factor for non-Hodgkin lymphoma or multiple myeloma.

For bladder cancer, two moderately sized (> 20 exposed cases) studies used a relatively specific exposure assessment method (Pesch et al. 2000; Lynge et al. 2006). Pesch et al. (2000) observed odds ratios 1.0, 1.2, and 1.8 for the medium, high, and substantial exposure categories, respectively, but the pattern was more variable in the nested case-control study by Lynge et al. (2006) in which duration of dry cleaning work was used as the exposure measure. Regarding smoking as an alternative explanation for bladder cancer, the case-control studies controlled for smoking. The studies of dry cleaning workers are also useful in that subjects are unlikely to have

been exposed to other occupational bladder carcinogens. In the small studies (< 10 exposed cases) of non-Hodgkin lymphoma, an approximate doubling of risk compared to the referent population was seen (Anttila et al. 1995; Boice et al. 1999; Calvert et al. 2011; Radican et al. 2008; Seldén and Ahlborg 2011), and a relative risk of 3.4 (95% CI: 0.7, 17.3) was seen in the highest cumulative exposure group in Seidler et al. (2007). Multiple myeloma is a relatively rare cancer, and results for multiple myeloma are based on a smaller set of studies and fewer observed cases than those for non-Hodgkin lymphoma. The largest of these studies, with 16 exposed cases, reported an odds ratio of 3.3 (95% CI: 1.2, 9.5) for the highest exposure group compared with the unexposed (Gold et al. 2010). For each of these three cancer types above, the epidemiologic data were considered to provide evidence suggestive of a causal association. For other sites, including esophageal, kidney, lung, liver, cervical, and breast cancer, results were more variable (data not shown). Studies of cancer in children exposed pre- or postnatally to tetrachloroethylene were inadequate to draw firm conclusions (Brown Dzubow et al. 2010).

Evaluation of experimental evidence of carcinogenicity

There is clear evidence of tetrachloroethylene carcinogenicity in rodents, provided by one oral gavage (NCI 1977) and two inhalation (JISA 1993; NTP 1986) cancer bioassays in sexually mature animals. No data were available on cancer risks in experimental animals exposed to tetrachloroethylene during early life stages. As summarized in Table 2, a number of factors were considered in evaluating the rodent carcinogenicity findings. These included statistical analyses to adjust for survival differences and cause of death, and mode-of-action analyses to inform judgments regarding human relevance of animal bioassay results and susceptible populations or lifestages (U.S. EPA 2005b).

Evaluation of rat tumors

The primary tumor finding in rats was a significant increase in the incidence of mononuclear cell leukemia (MCL) in both sexes in independent inhalation bioassays using the F344/N (JISA 1993; NTP 1986) or F344/DuCrj (JISA 1993) strain (see Figure 2). NTP's analyses of the tetrachloroethylene bioassay results revealed an increase in tumor incidence and severity in both sexes, and a shortened time to death with MCL in female rats. These results were affirmed by statistical analyses performed in a recent publication by Thomas et al. (2007) that noted tetrachloroethylene as one of only five of the 500 chemicals examined to produce "definitive" leukemia effects in both sexes of rats in NTP bioassays. The JISA (1993) study corroborated these results. There is a paucity of data in F344 rats on toxicokinetics or contributing metabolites or mechanisms to inform mode of action conclusions. Nonetheless, increases in hemolysis and bone marrow toxicity in NMRI mice following tetrachloroethylene exposure [Marth (1987); Marth et al. (1985a; 1985b; 1989); Seidel et al. (1992); Ebrahim (2001)] add some support to the biologic plausibility of the observed leukemic effects (NRC, 2010).

Kidney tumors, rare in male rats, were increased in a single bioassay (NTP 1986) (see Supplemental Material, Table S1). For rat kidney carcinogenesis, mechanistic data informative for the evaluation of tetrachloroethylene's carcinogenic mode of action were identified (summarized in Table 2). Tetrachloroethylene metabolites from the GSH conjugation pathway are thought to mediate mechanistic events (other than α2u-globulin nephropathy) contributing to renal carcinogenesis (Lash and Parker 2001). The glutathione conjugation metabolites TCVG, the cysteine conjugate TCVC, and the mercapturate NAcTCVC, are mutagenic in *Salmonella* tests, consistent with the observation that tetrachloroethylene tested positive for mutagenicity in the few studies in which products of the GSH conjugation metabolic pathway would have been

generated (Dekant et al. 1986; Dreessen et al. 2003; Vamvakas et al. 1987; Vamvakas et al. 1989a; Vamvakas et al. 1989b). However, *in vivo* evidence of genotoxicity in the kidney is limited to reports of modest effects following intraperitoneal (i.p.) exposures (Mazzullo et al. 1987) (Walles 1986). Evidence for α2u-globulin nephropathy did not meet EPA's criteria for establishing that renal tumors resulted from this mode of action (U.S. EPA 1991). Additionally, evidence of either cytotoxicity not associated with α2u-globulin accumulation, or of peroxisome proliferation, lacked specificity with regard to dose, sex and/or species.

Another rare rat tumor—brain glioma—was also increased in both sexes in a single bioassay (NTP 1986) (see Supplemental Material, Table S1). This study also reported increases in the rate of testicular interstitial cell tumors, a tumor type of high incidence in unexposed male F344 rats. Mechanistic or other data to inform the interpretation of the increases observed in rat brain and testicular tumors in the NTP bioassay were not identified.

Evaluation of mouse tumors

In mice, all three bioassays reported an increase in liver tumors following tetrachloroethylene exposure (NCI 1977; NTP 1986; JISA 1993) (see Figure 2). Statistical analyses of the gavage study (NCI 1977) revealed that the incidence of hepatocellular carcinomas or adenomas was significantly increased, and that tumor latency was significantly decreased. A significant association between increased mortality and tetrachloroethylene dose was seen, with liver tumors found in many mice dying prior to scheduled termination. Inhalation exposure to tetrachloroethylene induced significant, dose-related increases in the incidence of hepatocellular adenomas or carcinomas in both sexes of $B6C3F_1$ (NTP 1986) and Crj:BDF1 (JISA, 1993) mice.

The incidence of hepatocellular carcinomas that metastasized to the lungs was also significantly increased in the inhalation studies.

The mouse liver tumor evaluation also considered data on the hepatocarcinogenicity of tetrachloroethylene metabolites TCA and DCA. The primary urinary oxidative metabolite in rodents and humans, TCA, significantly increased the incidence of liver tumors in male and female B6C3F$_1$ mice exposed via drinking water for 52−104 weeks (Bull et al. 1990; Bull et al. 2002; DeAngelo et al. 2008; Herren-Freund et al. 1987; Pereira 1996). Liver tumor incidence increased with increasing TCA drinking water concentrations (Bull et al. 1990; Bull et al. 2002; DeAngelo et al. 2008; Pereira 1996). Tumors in TCA-exposed animals developed rapidly and significant increases were evident in less-than-lifetime studies of 82 or fewer weeks. Additionally, TCA was hepatocarcinogenic in mice when coadministered in the drinking water for 52 weeks with the tetrachloroethylene metabolite DCA (Bull et al. 2002). DCA alone also causes liver cancer in mice (Bull et al. 1990; Daniel et al. 1992; DeAngelo et al. 1999; Herren-Freund et al. 1987).

The issue of whether TCA can solely account for the hepatocarcinogenicity of tetrachloroethylene was addressed using toxicokinetic analyses, since the hepatocarcinogenic potencies of TCA and tetrachloroethylene have not been directly compared in a single rodent bioassay. EPA's analysis found that a wide range of possible contributions of TCA to tetrachloroethylene carcinogenicity, from as little as 12% to as much as 100%, is consistent with the available data [see Appendix C of US EPA (2012a)]. A more precise quantitative estimate of the relative contribution of TCA to tetrachloroethylene-induced liver tumors requires an appropriately designed study to better control for experimental variability in kinetics (e.g.,

dosing patterns in drinking water, metabolism, bioavailability) and dynamics (e.g., background tumor rates).

For hepatocarcinogenesis, a key issue was whether evidence for peroxisome proliferation is sufficient to establish the PPARα activation mode of action. The relevance to humans of tumors induced by this mode of action has been debated [see (Corton 2008; Guyton et al. 2009; Klaunig et al. 2003; Rusyn et al. 2006; Rusyn and Corton 2012)]. For instance, a dissenting opinion provided in the NRC peer review stated that "the weight of evidence strongly favors a key role of PPARα activation in tetrachloroethylene-induced hepatocarcinogenesis in mice; furthermore, this mode of action lacks relevance for human hepatocarcinogenesis" [refer to Appendix B; NRC (2010)]. However, the NRC peer review committee as a whole did not support these conclusions, stating in their rebuttal that many gaps in knowledge remain with regard to the hepatocarcinogenic mechanisms of tetrachloroethylene. Tetrachloroethylene-specific experiments have provided evidence that peroxisomal markers are increased, but at dose levels (1,000 mg/kg-day) exceeding those causing liver toxicity, proliferation, or carcinogenicity [Philip et al. (2007); Odum et al. (1988)]. In particular, Philip et al. (2007) reported that CYP4A, a marker of PPARα-activation, was only increased in Swiss Webster mice at the highest tetrachloroethylene dose (1,000 mg/kg-day) and at the earliest (7 days) but not later time points. In contrast, the study reported a robust dose-dependent proliferative response that persisted for 14−30 days post exposure at 150, 500, and 1,000 mg/kg-day levels of tetrachloroethylene. Liver toxicity and repair has been reported at lower doses in other studies in the $B6C3F_1$ strain [e.g., at 100 mg/kg-day in Schumann et al. (1980)]. Moderate increases in peroxisome proliferation have been reported in rats (Odum et al. 1988), a species insensitive to tetrachloroethylene

hepatocarcinogenicity. In total, these findings indicate that the modest peroxisome proliferative response to tetrachloroethylene may lack specificity with respect to species, tissue, and dose. The temporal sequence of events also remains to be established. Given these limitations, the database of tetrachloroethylene-specific studies was judged insufficient to demonstrate a causative role of this effect in hepatocarcinogenesis by tetrachloroethylene (U.S. EPA, 2012a). Tetrachloroethylene and/or its metabolites have been shown to induce a number of other mechanistic events that may also contribute to carcinogenicity, including mutagenicity, alterations in DNA methylation, and oxidative stress (U.S. EPA, 2012a).

In addition to mouse liver tumors, hemangiosarcomas or hemangiomas of the liver, spleen, fat, and subcutaneous skin were reported in male mice in one inhalation study (JISA 1993) (see Supplemental Material, Table S2). This mouse tumor type was not reported in the NCI oral gavage bioassay (NCI 1977), and no increase was reported in the NTP inhalation bioassay (NTP 1986). Mechanistic or other data to inform the interpretation of this tumor type were not identified.

Conclusions on carcinogenic hazard

Supported by the analyses described above, and following EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA 2005a) tetrachloroethylene is characterized as "likely to be carcinogenic to humans" (U.S. EPA 2012a). This characterization is based on suggestive evidence of carcinogenicity in epidemiologic studies and conclusive evidence that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, increases tumor incidence (JISA, 1993; NTP, 1986; NCI, 1977). The specific carcinogenic active moiety(ies) and mode(s) of action are not fully characterized, and the

hypothesis that mutagenicity contributes to the tetrachloroethylene carcinogenesis is not ruled out, particularly for kidney carcinogenicity. No data were available on cancer risks in animals exposed to tetrachloroethylene during early lifestages.

Non-cancer toxicity

EPA's analysis identified the central nervous system, kidney, liver, immune and hematologic systems, and development and reproduction, as target organs of tetrachloroethylene toxicity (U.S. EPA, 2012c). The supporting evidential database for each endpoint was variable, with few or limited studies of the relationship of effect with dose at low exposures for many endpoints. Neurotoxicity was supported by a considerable database of human, animal and mechanistic studies. Additionally, neurological effects were generally observed at lower tetrachloroethylene concentrations compared with other non-cancer health effects. Further, both the 2004 peer consultation workshop (U.S. EPA 2004) and the 2010 NRC peer review (National Research Council 2010) affirmed the conclusion that neurotoxicity is a sensitive endpoint since these effects were observed at lower concentrations and had substantial evidential support. The human and animal neurotoxicity findings supporting EPA's conclusions concerning tetrachloroethylene neurotoxicity are summarized below.

Neurotoxicity

Human studies regarding neurotoxicological hazard

The three primary neurological domains most consistently associated with subchronic or chronic tetrachloroethylene exposure in human studies were vision, visuospatial memory, and neuropsychological function (e.g., reaction time). Occupational and residential exposure studies support an association of visual deficits following chronic tetrachloroethylene exposure. Deficits

in color vision, relative to unexposed study participants, were observed in an occupational study (Cavalleri et al., 1994) and in a residential study (Schreiber et al., 2002). In a longitudinal follow-up study to Cavalleri et al (1994), Gobba et al (1998) reported that there was a worsening in the color vision in workers (self-comparison) that were exposed to higher levels of tetrachloroethylene. In the dry-cleaning facilities, color vision deficits, reported as a color confusion index (CCI) (Iregren et al. 2002), were significantly greater in exposed workers than in unexposed controls, with mean CCI scores of 1.143 and 1.108, respectively ($p = 0.03$) (Cavalleri et al., 1994). An additional 6% ($p < 0.01$) deterioration in mean CCI score relative to their previous score (reported in Cavalleri et al., 1994) was seen in workers exposed to increasing concentration of tetrachloroethylene (median of 1.67 – 4.35 ppm) two years later in a follow-up study of the same worker population (Gobba et al., 1998). Schreiber et al. (2002) reported lower CCI scores, in comparison to a non-exposed residential group, for adult and child residents living within a close proximity to dry cleaners with mean CCI scores of 1.33 in exposed and 1.20 in control ($p = 0.26$). In the same study no difference in CCI scores were observed in daycare workers working in a daycare center next to a dry cleaner in comparison to daycare workers in buildings with no tetrachloroethylene exposure. Two studies did not observe changes in color vision with tetrachloroethylene exposure, but were limited by no exposure characterization (Sharanjeet-Kaur et al. 2004) or by use of less sensitive color vision testing (Nakatsuka et al. 1992). In addition, deficits in visual contrast sensitivity relative to unexposed study participants were reported in the two residential populations living or working in buildings co-located with dry cleaners (Schreiber et al. 2002; Storm et al. 2011). For visual contrast sensitivity, there was a decreasing trend ($p < 0.05$) in the residential population achieving the maximum contrast

sensitivity score at 6 cycles per degree (cpd) with 28.3% in the referent group in comparison to 8.3% in the highest exposed (> 100 μg/m^3) group (Storm et al., 2011).

Associations between exposure and visuospatial memory were also reported in each of the studies that examined this measure in humans. These associations (increased response times or cognition errors) were reported in occupational (Echeverria et al. 1994; Echeverria et al. 1995; Seeber 1989) and residential studies (Altmann et al. 1995) related to dry-cleaning tetrachloroethylene exposure. In the occupational studies (Echeverria et al., 1995; 1994; Seeber, 1989) cognition errors ranged from 4-30% at exposures of 12-23 ppm, depending on the subtest that was used. In the residential study (Altmann et al., 1995), visual memory and cognitive function scores were 15% lower in individuals with a mean exposure of 0.7 ppm tetrachloroethylene compared to unexposed individuals. No studies specifically examined visuospatial memory in children.

For neuropsychological function, two studies reported 10-20% increases in simple reaction times, in comparison to an unexposed group, in tetrachloroethylene-exposed occupational (Ferroni et al. 1992) and residential (Altmann et al., 1995) settings. However, another occupational study reported 16% improvement in simple reaction time in comparison to unexposed individuals (Lauwerys et al. 1983).

Animal studies of neurotoxicological effects

Animal studies of subchronic tetrachloroethylene exposure also observed changes in visual function, cognitive function, and reaction time. In rats, acute inhalation exposure to tetrachloroethylene resulted in changes in visual evoked potentials (Boyes et al. 2009; Mattsson et al. 1998). In one subchronic exposure study (Mattsson et al. 1998), a significant increase in

amplitude and latency was observed in one peak of the visual evoked potential responses from a flash stimulus at 5,424 mg/m^3, but histopathological lesions were not observed in the examination of central and peripheral brain structures [e.g., visual cortex, optic nerve] of the visual system.

Significant changes in the motor activity domain as measured by increased reaction time, increased number of false alarms, and decreased trial completions in a signal detection task (measures of decreased attention) were reported at 6,782 mg/m^3 or higher in an acute exposure study in rats (Oshiro et al. 2008). Additionally, deficits in operant tasks that test cognitive performance were seen in rats and mice following acute oral (Warren et al. 1996) and *i.p.* (Umezu et al. 1997) exposures to tetrachloroethylene. These findings support evidence of deficits in cognition and memory associated with tetrachloroethylene exposure in humans. However, no animal studies to date have evaluated the persistence of cognitive performance deficits from acute or chronic tetrachloroethylene exposure.

Observed changes in brain weight, DNA/RNA, and neurotransmitter levels in experimental animals are consistent with evidence of neurobehavioral effects of tetrachloroethylene exposure in humans. Brain DNA, RNA, protein levels, and lipid composition were altered following tetrachloroethylene inhalation. Changes were observed in the cerebellum, the hippocampus, and the frontal cortex in sexually mature animals (Rosengren et al. 1986; Savolainen et al. 1977a; Savolainen et al. 1977b; Wang et al. 1993), as well as after gestational exposure (Kyrklund and Haglid 1991; Nelson et al. 1979).

Conclusions regarding non-cancer hazard

EPA's analysis identified the central nervous system, kidney, liver, immune and hematologic systems, and development and reproduction, as target organs of tetrachloroethylene toxicity (U.S. EPA, 2012c). Neurotoxicity was identified as among the most sensitive outcomes, occurring at low exposures. The assessment of the neurotoxicity studies drew conclusions through an examination of affected domains (e.g., cognition, vision, motor activity). Human and experimental animal studies provided complementary evidence regarding the association of neurobehavioral deficits and tetrachloroethylene exposure. Studies of humans exposed by inhalation suggest that chronic tetrachloroethylene exposure can result in decrements in vision, visuospatial memory, and possibly other aspects of cognition and neuropsychological function, including reaction time. Animal studies provide substantial support for associations of tetrachloroethylene exposure with effects in these domains of neurotoxicity.

Summary

Tetrachloroethylene is a widespread contaminant that is present in ambient air, indoor air, soil, drinking water and groundwater. Once exposed, humans and laboratory animal species rapidly absorb tetrachloroethylene. Tetrachloroethylene is then distributed to tissues via systemic circulation, metabolized, and excreted primarily in breath as unchanged tetrachloroethylene or CO_2, or in urine as metabolites. The role of metabolism in the toxicity of tetrachloroethylene was informed by development and application of an updated PBPK model (Chiu and Ginsberg 2011). Low oxidative metabolism was predicted in humans whereas GSH conjugation metabolism is more uncertain, and may be high, low, and/or highly variable. These PBPK model predictions

informed the extent and nature of metabolism in different target tissues, and the extrapolation across species and routes of exposure.

Following EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA 2005a), tetrachloroethylene is "likely to be carcinogenic to humans" by all routes of exposure. This characterization is based on suggestive evidence of carcinogenicity in epidemiologic studies and conclusive evidence that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, increases tumor incidence (JISA, 1993; NTP, 1986; NCI, 1977).

Neurotoxicity is identified as a sensitive outcome following either oral or inhalation exposure to tetrachloroethylene in humans and experimental animals. Associations between exposure and neurotoxic outcomes have been reported by human controlled exposure, occupational, and residential studies, as well as experimental animal studies, providing evidence that tetrachloroethylene exposure results in visual changes, increased reaction time, and decrements in cognition.

EPA's analysis approaches and conclusions are consistent with multiple sets of peer reviewer recommendations (U.S. EPA 2012a). Additionally, the use of evidence tables, narrative syntheses, and other aspects of the assessment approach were in accord with later NRC recommendations for improving IRIS assessments (National Research Council 2011). The International Agency for Research on Cancer (IARC) recently classified tetrachloroethylene as probably carcinogenic to humans (Group 2A) based on sufficient evidence in animals and limited evidence in humans, consistent with EPA's conclusion (Guha et al. 2012). Finally, studies of the health effects of tetrachloroethylene published since U.S. EPA's assessment

continue to report associations with neurological outcomes, including studies of illicit drug use (Aschengrau et al. 2011), mental illness (Aschengrau et al. 2012), visual effects (Getz et al. 2012), visuospatial functioning, learning and memory, motor, attention, and mood (Janulewicz et al. 2012), and Parkinson's disease (Goldman et al. 2012). Similarly, studies of tetrachloroethylene exposure and cancer continue to add support for human tumor sites identified in the U.S. EPA assessment (Christensen et al. 2013; Lipworth et al. 2011; Ruder et al. 2013; Vizcaya et al. 2013; Vlaanderen et al. 2013).

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Table 1. Results of epidemiology studies of tetrachloroethylene and bladder cancer, non-Hodgkin lymphoma, or multiple myeloma using higher quality exposure assessment methodology.

Reference, Population and Design	Exposure Surrogate	Bladder Cancer RR (95% CI) [n][a]	non-Hodgkin Lymphoma RR (95% CI) [n][a]	Multiple Myeloma RR (95% CI) [n][a]
Cohort Studies				
Antilla et al. (1995) Finland: biological monitored workers (SIR), blood tetrachloroethylene	Any tetrachloroethylene	not reported	3.76 (0.77, 11.0) [3]	(expected = 0.38) [0]
Boice et al. (1999) United States: aerospace workers (SMR, RR), job exposure matrix	Any routine exposure to tetrachloroethylene	0.70 (0.09, 2.53) [2]	1.70 (0.73, 3.34) [8]	0.40 (0.01, 2.25) [1]
	Duration, among those with routine or intermittent exposure to tetrachloroethylene:			
	No routine or intermittent exposure	not reported	1.0 (referent) [32]	1.0 (referent) [24]
	< 1 year		1.25 (0.43, 3.57) [4]	0.46 (0.06, 3.48) [1]
	1-4 years		1.11 (0.46, 2.70) [6]	1.13 (0.38, 3.35) [4]
	≥ 5 years		1.41 (0.67, 3.00) [10]	0.24 (0.03, 1.84) [1]
Blair et al. (2003) United States laundry and dry-cleaning workers (SMR), union records	Little to no tetrachloroethylene exposure	1.4 (0.4, 3.2) [5]	not reported	not reported
	Medium to high tetrachloroethylene exposure	1.5 (0.6, 3.1) [7]		
Lynge et al. (2006) Sweden, Denmark, Finland, Norway: nested case-control, census occupation codes and pension data/questionnaires	Dry cleaner job title	1.44 (1.07, 1.93) [93]	not studied	not studied
	Employment duration (dry cleaner):		not studied	not studied
	Never held job in dry cleaning	1.0 (referent) [188]		
	< 1 year	1.50 (0.57, 3.96) [6]		
	2-4 years	2.39 (1.09, 5.22) [10]		
	5-9 years	0.91 (0.52, 1.59)[17]		
	> 10 years	1.57 (1.07, 2.29) [54]		
	Unknown duration	1.97 (0.64, 6.05) [6]		

Reference, Population and Design	Exposure Surrogate	Bladder Cancer RR (95% CI) [n][a]	non-Hodgkin Lymphoma RR (95% CI) [n][a]	Multiple Myeloma RR (95% CI) [n][a]
Radican et al. (2008) United States: aircraft maintenance workers (RR-internal referent), job exposure matrix	Any tetrachloroethylene			
	Males	not reported	2.32 (0.75, 7.15) [5]	1.71 (0.42, 6.91) [3]
	Female	not reported	2.35 (0.52, 10.7) [2]	7.84 (1.43, 43.1) [2]
Seldén and Ahlborg (2011) Sweden: dry-cleaning workers (SIR), census occupation codes, questionnaire, and company-provided data pertaining to solvent use	Any tetrachloroethylene			
	Males	not reported	2.02 (1.13, 3.34) [15]	not reported
	Females	not reported	1.14 (0.68, 1.81) [18]	not reported
Calvert et al. (2011) United States: laundry and dry-cleaning workers (SMR), union employment records (tetrachloroethylene-only exposure based on history of solvent use by shops)	Any tetrachloroethylene	not reported [0]	2.46 (0.90, 5.36) [6]	not reported
Case-control Studies				
Aschengrau et al. (1993), United States (Massachusetts): residential history, ordinal estimate of tetrachloroethylene-contaminated water from exposure model	Any tetrachloroethylene	1.39 (0.67, 2.91) [13]	not studied	not studied
	Any tetrachloroethylene above the 90th percentile Relative Delivered Dose	4.03 (0.65, 25.10) [4]	not studied	not studied
Pesch et al. (2000) (Germany): job and task exposure matrix	Any tetrachloroethylene (males):		not studied	not studied
	Medium exposure)	1.0 (0.7, 1.5) [37]		
	High exposure	1.3 (0.8, 1.7) [47]		
	Substantial exposure	1.8 (1.1, 3.1) [22]		
Miligi et al. (2006)[b], Costantini et al., (2008)[b] Italy: job exposure matrix	Any tetrachloroethylene:	not studied		
	Very low/low intensity		0.6 (0.3, 1.2) [18] [c]	not reported [3]
	Medium/high intensity		1.2 (0.6, 2.5) [14] [c]	not reported [2]

Reference, Population and Design	Exposure Surrogate	Bladder Cancer RR (95% CI) [n][a]	non-Hodgkin Lymphoma RR (95% CI) [n][a]	Multiple Myeloma RR (95% CI) [n][a]
Seidler et al. (2007) Germany: job exposure matrix	Tetrachloroethylene, cumulative exposure, ppm-years:	not studied		
	0		1.0 (referent) [667]	1.0 (referent) [33]
	> 0 to ≤ 9.1		1.1 (0.5, 2.3) [16][d]	1.8 (0.5, 6.7) [3]
	> 9.1 to ≤ 78.8		1.0 (0.5, 2.2) [14] [d]	[0]
	> 78.8		3.4 (0.7, 17.3) [6] [d]	[0]
Gold et al. (2010) United States: all jobs held >12 months, job exposure matrix	Any tetrachloroethylene	not studied	not studied	1.5 (0.8, 2.9) [16]
	Cumulative tetrachloroethylene exposure (ppm-wks)			
	0	not studied	not studied	1.0 [164]
	1−353			0.3 (0.04, 3.0) [1]
	354−1,430			0.5 (0.1, 4.4) [1]
	1,431−4,875			1.5 (0.4, 5.4) [4]
	4,876−13,500			3.3 (1.2, 9.5) [10]

[a]n = number of exposed cases. [b]Both the Miligi et al. (2006) and Costantini et al., (2008) are of the Italian Multicenter Case–control Study on Hematolymphopoietic Malignancies and Exposure to Solvents and Pesticides. Miligi et al. (2006) report odds ratios for non-Hodgkin lymphoma and tetrachloroethylene; Costantini et al. (2008) report odds ratios for multiple myeloma and tetrachloroethylene. [c]Includes patients with non-Hodgkin lymphoma and chronic lymphocytic leukemia. [d]Includes patients with non-Hodgkin and Hodgkin lymphoma.

CI = confidence interval; ppm = parts per million; RR = relative risk; SIR = standardized incidence ratio; SMR = standardized mortality ratio; yrs = years.

Table 2. Summary of factors considered in evaluating carcinogenicity findings in experimental animals.

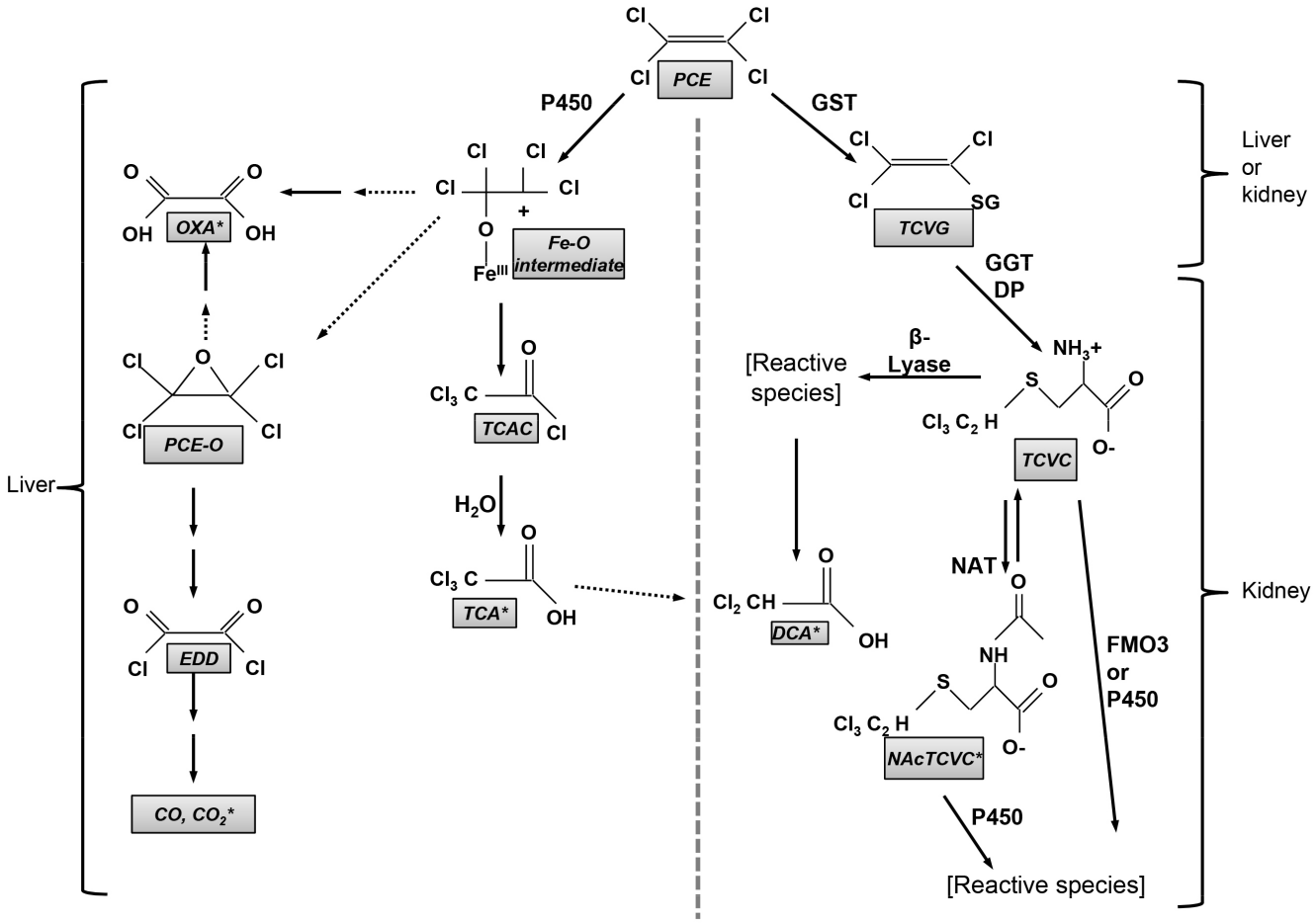
Tumor type	Incidence (dose, sex, strain, route)	Tumor latency, severity, mortality, and background rate	Toxicokinetic information	Mode of action (MOA) information
Rat mononuclear cell leukemia	Significant increases in both sexes of F344/N (NTP 1986) and F344/DuCrj (JISA 1993) strains; dose-dependent increase in F344/DuCrj males (JISA 1993)	In the NTP study: decreased latency in females; increased severity in both sexes; background rate 56% in males, 36% in females. In the JISA study: decreased latency in both sexes; background rate of ~ 20% in both sexes	No information available concerning active moiety(ies)	None hypothesized; studies demonstrating hemolysis and bone marrow toxicity in mice add some support to the biologic plausibility
Mouse hepatocellular tumors	Significant, dose-dependent increases in both sexes of $B6C3F_1$ (NTP 1986) and Crj:BDF1 (JISA, 1993) strains with inhalation exposures; no continued increase with increasing dose in gavage study of $B6C3F_1$ strain (NCI, 1977)	Decreased latency; increased mortality; increased metastases in inhalation studies; background rate of ~ 30% in males, ~ 8% in females	The metabolites TCA and DCA are mouse hepatocarcinogens, alone and in combination	Evidence is insufficient for the hypothesized MOAs evaluated: PPARα activation; mutagenicity; alterations in DNA methylation; oxidative stress secondary to cytotoxicity.
Mouse hemangiomas, hemangiosarcomas	Significant, dose-dependent increases in male Crj:BDF1 mice (JISA, 1993)	Background rate of 2-4% in both sexes; decreased latency	No information available concerning active moiety(ies)	None hypothesized
Rat kidney tumors	Significant trend in one bioassay (NTP, 1986) compared to historical controls	Low background rate (1/549 among historical controls for facility; ~ 0.2% in 1968 untreated controls in the NTP program)	Glutathione conjugation metabolites are likely contributors to renal carcinogenicity	Evidence is insufficient for the hypothesized MOA evaluated: α2u-globulin nephropathy did not meet EPA's criteria for establishing this MOA; evidence of either a) cytotoxicity not associated with α2u-globulin accumulation or b) peroxisome proliferation lacked specificity with regard to dose, sex and/or species; limited evidence of mutagenicity (+ Ames assays with GSH conjugation metabolites)

Figure Legends

Figure 1. Simplified tetrachloroethylene (PCE) metabolism scheme. Tetrachloroethylene is metabolized in humans and experimental animal species by both oxidation and GSH conjugation metabolic pathways, yielding numerous toxicologically active compounds (Lash and Parker 2001). Tetrachlorethylene metabolism yields the oxidative metabolites TCAC, which hydrolyses to yield TCA, and the epoxide tetrachloroethylene-O which decomposes in turn to EDD, CO and CO_2. Oxalic acid is also a product of tetrachloroethylene oxidation. GSH conjugation products include TCVG, the cysteine conjugate TCVC and the mercapturate NAcTCVC and its sulfoxidation products. DCA is likely produced via beta-lyase-mediated bioactivation, although TCA dechlorination may be an additional minor source.

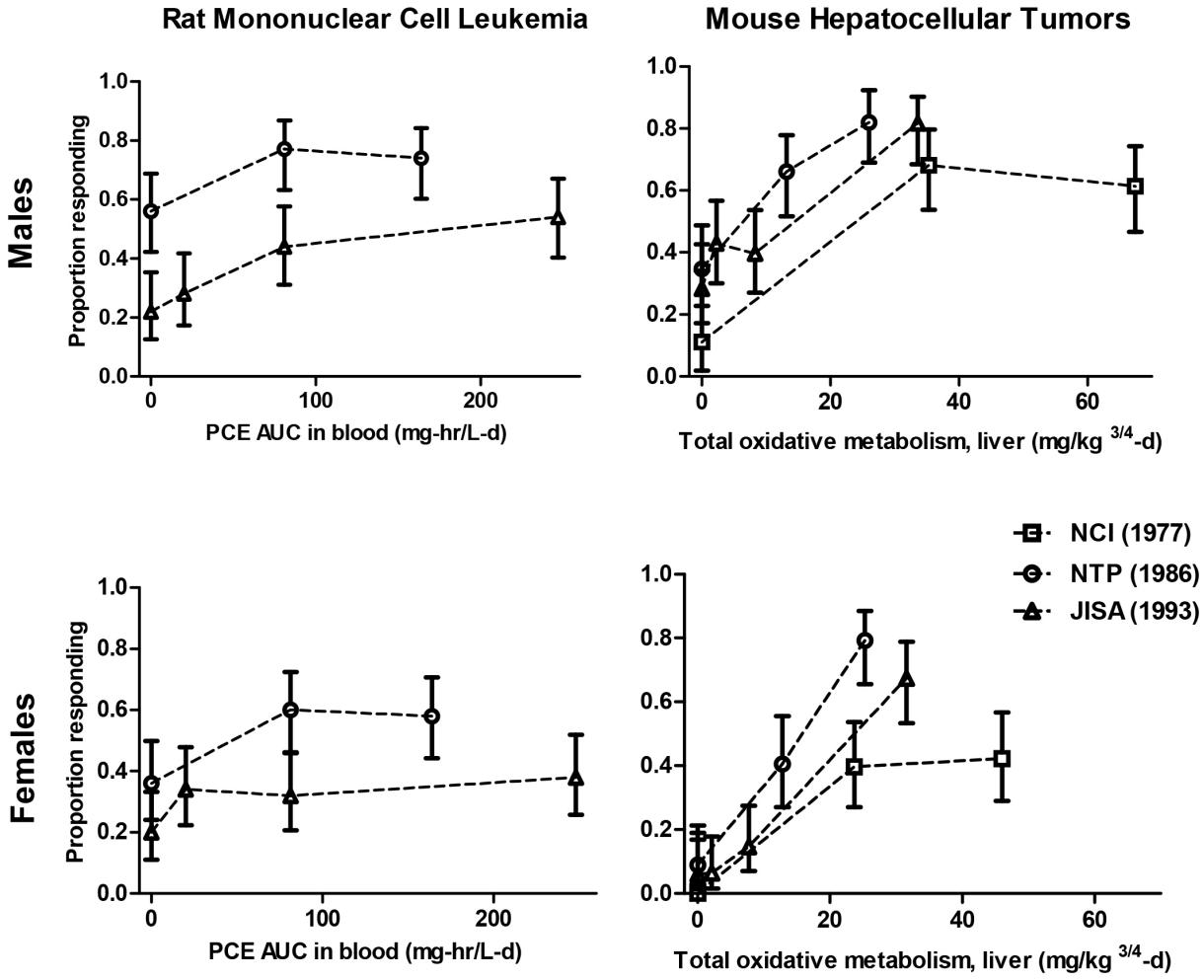
Figure 2. Dose-response relationships for rat mononuclear cell leukemias and mouse hepatocellular tumors in tetrachloroethylene bioassays. Three laboratories evaluated tetrachloroethylene in both mice and rats (oral gavage: NCI, 1977; inhalation: NTP, 1986; JISA, 1998). The Chiu and Ginsberg (2011) PBPK model was used to estimate internal dose for each site, allowing comparison of responses across routes of exposure. The best supported dose metric for mouse liver tumors was total oxidative metabolism, whereas that for rat MCLs was tetrachloroethylene area-under-the-curve (PCE AUC) in blood. The NCI (1977) study in Osborne-Mendel rats was judged inconclusive because of high rates of respiratory disease and mortality with tetrachloroethylene and thus rat data from this study are not presented. Survival-adjusted responses are presented as proportion responding (incidence/number at risk).

Figure 1.



* Metabolites identified in blood, urine, or breath following in vivo PCE exposure (rodent or human).

Figure 2.


Rat Mononuclear Cell Leukemia
Mouse Hepatocellular Tumors
Males
Females
Proportion responding
PCE AUC in blood (mg-hr/L-d)
Total oxidative metabolism, liver (mg/kg 3/4-d)
NCI (1977)
NTP (1986)
JISA (1993)
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
20
40
60